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An improved RP-HPLC method for determining nitroaromatics and nitramines in water

Thomas F. Jenkins, Paul H. Miyares and Marianne E. Walsh

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A protocol was developed for determining nitroaromatic and nitramine explosives by reversed-phase, high-performance liquid chromatography on an LC-18 column. The method employs dilution of an aqueous sample 1:1 with methanol, filtration through a 0.5-μm Millex-SR filter, separation on the LC-18 column using a 1:1 water-methanol eluent, and determination by UV-254 nm. A careful comparison was made with an earlier standard protocol, which uses separation on an LC-8 column with a 50:38:12 water-methanol-acetonitrile eluent. Overall, the new procedure provides better separation for a wider range of analytes and equivalent recovery for all analytes tested. The new procedure is particularly effective at separating TNT from tetryl, and it allows analysis of water and soil extracts using a single column and eluent combination. Reporting limits for HMX, RDX, TNB, DNB, NB, TNT, 2,4-DNT, 2,6-DNT, o-NT, m-NT, p-NT and 2,4,5-TNT ranged from 4 to 15 μg/L. A higher reporting limit of 44 μg/L was obtained for tetryl, apparently because of some instability in the methanol-water matrix. Analytical recovery averaged 99%, with standard deviations ranging from 1.6 to 5.1 μg/L (standard deviation for tetryl = 15.7 μg/L).

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PREFACE

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ABBREVIATIONS

DNB	1,3-dinitrobenzene
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
NB	nitrobenzene
NG	nitroglycerine
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SEX	octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine
TAX	hexahydro-1-(N)-acetyl-3,5-dinitro-1,3,5-triazine
TNB	1,3,5-trinitrobenzene
TNT	2,4,6-trinitrotoluene
Tetryl	methyl-2,4,6-trinitrophenylnitramine
m-NT	meta-nitrotoluene
o-NT	ortho-nitrotoluene
p-NT	para-nitrotoluene
2-Am-DNT	2-amino-4,6-dinitrotoluene
4-Am-DNT	4-amino-2,6-dinitrotoluene
2,4-DAm-NT	2,4-diamino-6-nitrotoluene
2,6-DAm-NT	2,6-diamino-4-nitrotoluene
2,4-DNT	2,4-dinitrotoluene
2,6-DNT	2,6-dinitrotoluene
2,4,5-TNT	2,4,5-trinitrotoluene
AAP	Army Ammunition Plant
CRL	Certified Reporting Limits
MDL	Method Detection Limits
RP-HPLC	Reversed-Phase, High-Performance Liquid Chromatography
RSD	Relative Standard Deviation
SARM	Standard Analytical Reference Material
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
UV	Ultraviolet

An Improved RP-HPLC Method for Determining Nitroaromatics and Nitramines in Water

THOMAS F. JENKINS, PAUL H. MIYARES AND MARIANNE E. WALSH

INTRODUCTION

Several years ago CRREL was asked by the Large Caliber Weapons Systems Laboratory to assess the methods available for simultaneously determining HMX, RDX, TNT and 2,4-DNT in munitions wastewater and to develop a specific protocol that could be used to monitor waste streams at Army Ammunition Plants. In response CRREL developed a protocol based on Reversed-Phase, High-Performance Liquid Chromatography (RP-HPLC) (Jenkins et al. 1984, 1986). This method was subsequently evaluated by means of a collaborative test (Bauer et al. 1986) and later accepted by the Association of Official Analytical Chemists as the standard method for the determination of TNT, RDX, HMX and 2,4-DNT in wastewater and groundwater (AOAC 1986). The four analytes were separated on an LC-8 column using a mobile phase consisting of 50% water, 38% methanol and 12% acetonitrile, with determination by a UV detector at 254 nm.

More recently, CRREL was asked by the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) to assess methods appropriate for determining explosive residues in soil. The analytes of interest were specified as the original four (HMX, RDX, TNT and 2,4-DNT) plus TNB (1,3,5-trinitrobenzene), DNB (1,3-dinitrobenzene) and tetryl (methyl-2,4,6-trinitrophenylnitramine).

Again, a protocol was developed that relied on RP-HPLC to separate the seven analytes; however, an LC-18 column, using a mobile phase composed of 1:1 V/V water–methanol, was chosen. LC-18 was selected over LC-8 for this application because it was not possible to achieve baseline separation of tetryl and TNT on LC-8 using either the mobile phase developed for the original water method or any other binary or tertiary mixture of water, methanol and acetonitrile. The LC-18 achieved baseline separation for all seven analytes under isocratic conditions in a run time of only 12 minutes (Jenkins and Walsh 1987). CRREL recommended confirming analyte identities by use of a separation on an LC-CN column, also with a mobile phase composed of 1:1 V/V water–methanol.

USATHAMA and the Corps of Engineers are conducting a number of studies at current and former Army installations to see if they are contaminated with explosives. Both water and soil samples are often analyzed. Currently, the agencies using the methodology developed at CRREL for explosives analysis require water analyses on an LC-8 column and soil analyses on an LC-18 column. Clearly, it would be more efficient and cost-effective to conduct both analyses on the LC-18 column if a protocol for water analysis based on this separation could be developed. Before this change is adopted, however, the new protocol must be thoroughly tested to ensure that it performs as well as the earlier LC-8 based method, which has undergone a rigorous collaborative test.

Our objective here was to develop a protocol using an LC-18 separation to determine HMX, RDX, TNB, DNB, tetryl, TNT, and 2,4-DNT in water. Specifically, we wanted to define the methodological steps, quantify the figures of merit for the overall method, test the method with real and spiked samples and test the ability of the method to resolve the analytes of interest from the most likely po-

tential interferences. In addition, the method was also tested for a number of other potential analytes including: 2,6-DNT, the three isomers of nitrotoluene (o-NT, m-NT, p-NT), nitrobenzene (NB), nitroglycerine (NG), benzene and 2-amino-4,6-dinitrotoluene (2-Am-DNT) (one of the principal microbiological decomposition products of TNT). Analyses for some of these analytes have been required for surveys conducted under the auspices of the U.S. Army Engineer Division, Missouri River.

EXPERIMENTAL METHODS

Instrumentation

All the RP-HPLC determinations were made on a modular system composed of the following:

1. A Perkin-Elmer Series 3 or Spectra-Physics SP8810 pump.

2. A Dynatech Precision Sampling Model LC-241 autosampler containing a Rheodyne Model 7010A sample loop injector.

3. Either a Spectra-Physics Model SP8300 UV-254 nm fixed wavelength detector, a Perkin-Elmer LC-65T variable wavelength UV detector or a Spectra-Physics Model SP8490 variable wavelength detector set at 254 nm.

4. A Hewlett Packard 3393A digital integrator equipped with a Hewlett Packard 9114B disk drive.

5. A Linear Model 500 strip chart recorder.

A diagram of the protocol is shown in Figure 1. Results from the integrator were obtained in the peak height mode, which demonstrated much better reproducibility at low levels than automated peak area measurements.

The analytes were separated on either a 25-cm x 4.6-mm (5-μm) Supelco LC-8 or LC-18 reversed-phase column. The LC-8 column was eluted with 1.5 mL/min of 50:38:12 water/methanol/acetonitrile (V/V/V). The LC-18 column was eluted with 1.5 mL/min of 1:1 water/methanol (V/V). Samples were introduced by overfilling a 100-μL sampling loop. For analyte confirmation (Jenkins and Grant 1987) we also obtained retention times on an LC-CN column using 1:1 water/methanol (V/V).
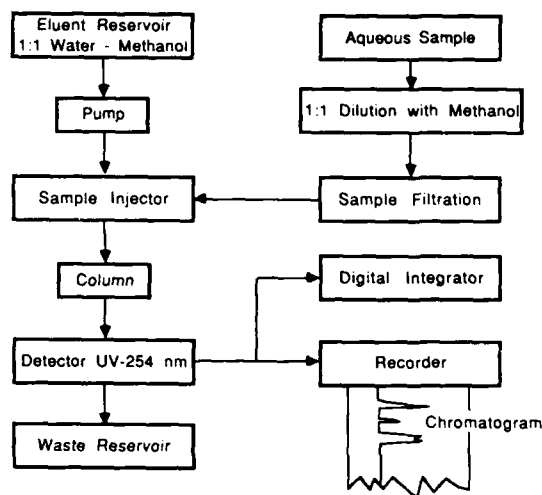
Figure 1. *Flow diagram of RP-HPLC protocol from sample preparation through sample analysis and data recovery.*

Chemicals

Analytical standards for HMX, RDX, TNB, DNB, tetryl, TNT, 2,4-DNT, 2,6-DNT, NG and NB were prepared from Standard Analytical Reference Materials (SARM's) obtained from USATHAMA, Aberdeen Proving Ground, Maryland. Standards for SEX, TAX, 2-Am-DNT, 4-Am-DNT, 2,4-DAm-NT and 2,6-DAm-NT were obtained from Dr. David Kaplan, U.S. Army Natick Laboratories (Natick, Massachusetts). Standards for o-NT, m-NT and p-NT were obtained from Eastman Chemicals. Standards (except NG) were dried to constant weight in a vacuum desiccator over dry calcium chloride in the dark; NG is supplied at a known concentration in an acetone solution and was used in this form.

The methanol and acetonitrile used to dilute samples or prepare the RP-HPLC eluents were either Mallinckrodt ChromAR HPLC or Baker HPLC grade solvents. Water used for preparation of eluents was purified using a Milli-Q Type 1 Reagent Grade Water System (Millipore Corporation). Water used to dilute wastewater samples was obtained from a deep groundwater aquifer in Hanover, New Hampshire. Munitions samples used in method comparison studies were obtained from the Iowa Army Ammunition Plant, Middletown, Iowa.

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We prepared the mobile phases by combining the proper portions of individual chemicals and vacuum filtering through a Whatman CF-F microfiber filter to remove particulates and degas the eluent.

Preparation of calibration standards

We prepared individual stock standards of HMX, RDX, TNB, DNB, tetryl, TNT, 2,4-DNT, 2,6-DNT, NB, 2-Am-DNT, o-NT, m-NT, p-NT, 4-Am-DNT, 2,4-DAm-NT, 2,6-DAm-NT, benzene, toluene, SEX and TAX by carefully weighing out about 100 mg of each dried standard material to the nearest 0.01 mg, transferring to individual 250-mL volumetric flasks and diluting to volume with acetonitrile. The stock standard of 2,4,5-TNT was made in a similar manner, except 21 mg was dissolved in 200 mL. Flasks were wrapped with Parafilm to retard evaporation and were stored at 4°C in the dark. We prepared the stock standard for NG by transferring the total contents of the sealed ampule (200 mg NG) to a 100-mL volumetric flask and bringing to volume with methanol.

Two combined analyte stock standards were prepared for linearity and reporting limit tests. Solution 1 was prepared by combining 4.00 mL of the HMX and RDX stock standards with 2.00 mL of the TNB, DNB, tetryl, TNT and 2,4-DNT stock standards in a 200-mL volumetric flask and bringing to volume with methanol. This solution contained about 8000 µg/L of HMX and RDX and about 4000 µg/L of TNB, DNB, tetryl, TNT and 2,4-TNT. Solution 2 was prepared in a similar manner using 2.00 mL of the HMX, RDX and 2,6-DNT stock standards, 1.25 mL of 2-Am-DNT and 1.00 mL of the TNT, TNB, DNB, o-NT, p-NT, m-NT and NB stock standards. Solution 2 contained about 4000 µg/L of HMX, RDX and 2,6-DNT, 2000 µg/L of TNT, TNB, DNB, o-NT, p-NT, m-NT and NB and about 2500 µg/L of 2-Am-DNT.

For linearity testing, we made up two separate sets of dilutions from each of solutions 1 and 2. For solution 1, the combined analyte stock standard was diluted 50:150 V/V with methanol. Further dilutions were prepared for solution 1 as shown in Table 1. For solution 2, the combined analyte stock standard was diluted 1:1 V/V with methanol. We prepared further dilutions as shown in Table 2.

Samples for certified reporting limit tests

Two combined stock standards (solutions 3 and 4) were prepared for Certified Reporting Limit (CRL) tests. For solution 3, 5.00 mL of the HMX, RDX, o-NT, m-NT and p-NT stock standards and 1.00 mL of the TNB, DNB, NB, TNT and 2,6-DNT stock standards were combined in a 100-mL volumetric flask and diluted to volume with methanol. This solution contained about 20,000 µg/L of HMX, the nitrotoluenes and RDX, and 4000 µg/L of TNB, DNB, NB, TNT and 2,6-DNT. We prepared sample J (Table 3) by diluting 10.0 mL of solution 3 with reagent grade water in a 100-mL volumetric flask. Further dilutions of sample J were prepared as shown in Table 3.

For solution 4, we combined 5.00 mL of the tetryl stock solution, 1.00 mL of the 2,4-DNT stock, and 4.00 mL of the 2,4,5-TNT stock in a

Table 1. Preparation of calibration standards from solution 1.

Standard	Aliquot of diluted standard (mL)	Size of volumetric flask (mL)	Approximate concentration (µg/L)	
			HMX, RDX	TNB, DNB, tetryl, TNT, 2,4-DNT
A	25	50	1000	500
B	25	100	500	250
C	10	100	200	100
D	5	100	100	50
E	5	200	50	25
F	1	100	20	10
G	10 *	100	10	5
H	5 *	100	5	2.5

* Aliquot of standard D.

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Table 2. Preparation of calibration standards from solution 2.

Standard	Aliquot of diluted standard (mL)	Size of volumetric flask (mL)	Approximate concentration (μg/L)		
			HMX, RDX 2,6-DNT	2,Am-DNT	TNB, DNB, TNT, o-NT p-NT, m-NT, NB
AA	50	100	1000	625	500
BB	10 *	25	400	250	200
CC	10 *	50	200	125	100
DD	10 *	100	100	60	50
EE	4 *	100	40	25	20
FF	2 *	100	20	12.5	10
GG	1 *	100	10	6.3	5

* Aliquot of diluted standard AA.

Table 3. Preparation of samples for reporting limit testing from solution 3.

Sample	Aliquot of sample J* (mL)	Size of volumetric flask (mL)	Approximate concentration (μg/L)	
			HMX, RDX o-, p-, m-NT	TNB, DNB, NB TNT, 2,6-DNT
J	—	—	2000	400
K	10	100	200	40
L	5	100	100	20
M	2	100	40	8
N	1	100	20	4
O	5 of K	100	10	2

* Dilutions made with reagent grade water.

100-mL volumetric flask and diluted to volume with methanol. Further dilutions of solution JJ were prepared as shown (Table 4) using reagent grade water.

Duplicate 5.00-mL aliquots of each sample (K-O and KK-OO) were diluted with 5.00 mL of methanol in individual glass scintillation vials. We mixed the solutions and filtered them through individual 0.5-μm Millex-SR disposable filters, discarding the first 3 mL and collecting the remainder. The concentrations of analytes were determined on LC-18 as described previously. We prepared and analyzed the samples on each of four days.

Determination of method detection limits

Method Detection Limits (MDL's) were determined by preparing ten aliquots each of two test solutions and processing each as described above for CRL tests. The first solution contained HMX and RDX at 32 μg/L and TNB, DNB, NB, TNT, 2,6-DNT and o-NT, n-NT, and p-NT at 20 μg/L. The second solution contained tetryl at 30 μg/L, and 2,4,5-TNT, 2,4-DNT and 2-Am-DNT at 20 μg/L. All determinations were made on one day on the LC-18 column under the conditions described above. MDL values were obtained according to the EPA protocol described elsewhere (Federal Register 1984).

RESULTS AND DISCUSSION

Retention times

The retention times and capacity factors on all three columns for the major analytes and the most probable interferences are com-

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Table 4. Preparation of samples for reporting limit testing from solution 4.

Sample	Aliquot of sample JJ* (mL)	Size of volumetric flask (mL)	Approximate concentration (µg/L)		
			Tetryl	2,4-DNT	2,4,5-TNT
JJ	—	—	2000	400	400
KK	10	100	200	40	40
LL	5	100	100	20	20
MM	2	100	40	8	8
NN	1	100	20	4	4
OO	5 of KK	100	10	2	2

*Dilutions made with reagent grade water.

Table 5. Retention times and capacity factors for major analytes and potential interferences on LC-8, LC-18 and LC-CN columns.*

Substance	Retention time (min)			Capacity factor (k)		
	LC-8	LC-18	LC-CN	LC-8	LC-18	LC-CN
HMX	3.68	2.44	8.35	0.62	0.49	2.52
RDX	4.66	3.73	6.15	1.05	1.27	1.59
TNB	5.42	5.11	4.05	1.39	2.12	0.71
DNB	6.21	6.16	4.18	1.74	2.76	0.76
TNT	8.16	8.42	5.00	2.59	4.13	1.11
2,4-DNT	9.01	10.05	4.87	2.97	5.13	1.05
Tetryl	7.88	6.93	7.36	2.47	3.23	2.11
NG	—	7.74	6.ͺͻ	—	3.72	1.53
NB	6.67	7.23	3.81	1.98	3.41	0.61
m-NT	11.19	14.23	4.45	3.93	7.68	0.88
p-NT	10.62	13.26	4.41	3.68	7.09	0.86
o-NT	10.13	12.26	4.37	3.46	6.48	0.84
2-Am-DNT	8.66	9.12	5.65	2.81	4.56	1.38
4-Am-DNT	8.81	8.88	5.10	2.88	4.41	1.15
SEX	3.00	2.40	5.07	0.32	0.46	1.14
TAX	3.28	2.78	3.70	0.44	0.70	0.56
2,4,5-TNT	—	8.44	5.89	—	4.15	1.49
2,4-DAm-NT	3.18	3.16	4.20	0.40	0.93	0.77
2,6-DAm-NT	3.00	2.39	3.70	0.32	0.46	0.56
2,6-DNT	9.25	9.82	4.61	3.07	4.99	0.95
Benzene	—	11.22	3.48	—	5.84	0.47
Toluene	—	23.0	3.93	—	13.02	0.66

* Eluents were 1:1 V/V water–methanol for LC-18 and LC-CN columns and 50:38:12 V/V/V water–methanol–acetonitrile for LC-8. Flow rate was 1.5 mL/min for all three columns.

pared in Table 5. The retention order is nearly identical for LC-8 and LC-18 (Fig. 2). However, compared to LC-18, we observed a different elution order on LC-CN, where the nitramines (HMX, RDX, SEX, TAX, tetryl) are retained longer than are the nitroaromatics (Table 5). Coelution of TNB and DNB as well as TNT and 2,4-DNT limits the utility of LC-CN as the primary analytical column, but the much greater retention of nitramines (HMX, RDX, tetryl, TAX, SEX) compared to either LC-8 or LC-18 makes LC-CN useful for

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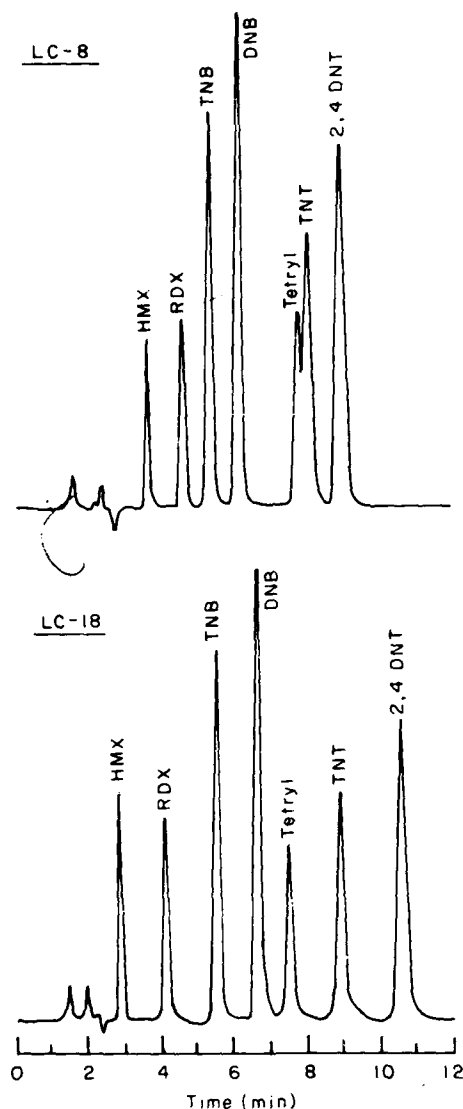
Figure 2. Comparison of retention time order and column separation performance of analytes for LC-8 (50:38:12 water/MeOH/AeV) versus LC-18 (50:50 water/MeOH).

provides excellent separation from TNT (about 1.5 minutes) and adequate separation from DNB (about 0.8 minutes). We were unable to adequately separate tetryl from TNT on LC-8 with either the standard eluent (50:38:12) or any other binary or tertiary combination of water, methanol and acetonitrile (Jenkins and Walsh 1987). The LC-18 column overall provides greater capacity factors and better separation than LC-8 for the majority of compounds tested. There appears to be no disadvantage to the separation achieved with the LC-18 column compared to LC-8 for any of the suite of potential contaminants tested. Experience indicates that the analytes most often observed in environmental analyses at contaminated Army sites are TNT, RDX, HMX, TNB and 2-Am-DNT. These five analytes are very well separated on the LC-18 column using the eluent described (Fig. 2). LC-18 also provides excellent separation of NB, the three isomers of nitrotoluene, benzene and 2,4-DNT from each other and from the major analytes of interest.

In a single determination, an analyst can simultaneously determine HMX, RDX, TNB, DNB, NB or tetryl, TNT, 2-Am-DNT, 2,4- or 2,6-DNT, benzene, and the three isomers of nitrotoluene under isocratic conditions in a run time of 18 minutes. Since the retention times of 2,4- and 2,6-DNT differ by only 0.2 minutes, large amounts of one isomer will mask small concentrations of the other. Thus when linearity and reporting limit tests were conducted, 2,4-DNT and 2,6-DNT were studied in separate sets of standards (Tables 1 and 2).

second column confirmations of analyte identities (Jenkins and Walsh 1987, Jenkins and Grant 1987).

A comparison of LC-8 and LC-18 indicates that both columns adequately separate the original suite of four analytes (HMX, RDX, TNT and 2,4-DNT) under isocratic conditions with approximately equivalent run times (about 10 minutes). Both columns also separate TNB and DNB from each other and from the other four analytes. For tetryl, LC-18

Sample dilution and filtration

Filtration experiments have indicated that some of the explosives are lost when aqueous samples are filtered through some types of disposable filter membranes (Jenkins et al. 1987, Walsh et al. 1988). Since filtration is necessary to protect expensive RP-HPLC columns, water should be diluted with a miscible organic solvent prior to filtration. This dilution with an organic solvent was shown to be very effective at eliminating analyte losses during filtration. Dilution of samples with a solvent also allows matrix matching of the sample composition to the RP-HPLC mobile phase. Matrix matching minimizes baseline disturbance ascribable to any difference in

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sample–eluent composition and produces more accurate determinations of early eluters such as HMX (Jenkins et al. 1984).

The analytical method developed for determining explosive residues in soil calls for an acetonitrile extraction followed by dilution 1:1 with water prior to RP-HPLC determination on an LC-18 column. For this method, the mobile phase is 1:1 water/methanol, but the injected sample is 1:1 water/acetonitrile. This difference is necessary in the soil method because acetonitrile was found to be a better extractant than methanol, particularly for HMX and RDX (Jenkins and Leggett 1985, Jenkins and Grant 1987), but the separation requires a methanol–water mobile phase to suitably separate the major analytes.*

For water analysis on LC-18, the water could be diluted with either methanol or acetonitrile prior to filtration. To see if it makes a difference which solvent is used, we diluted a combined analyte standard 1:100 with local groundwater and placed twenty 5.00-mL aliquots in individual 20-mL glass scintillation vials. The replicates were randomly divided into two sets of ten. One set was diluted 1:1 V/V with methanol, filtered through a 0.5-µm Millex-SR filter and determined on LC-18 as usual. Response factors for this set were obtained by analysis of a combined standard prepared by dilution of individual stock standards in methanol. The second set was processed in a similar manner, except acetonitrile was used to dilute the samples and as the solvent for preparation of the combined stan-

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* Use of a water–acetonitrile mobile phase results in co-elution of HMX and RDX.

dard. Results for the six analytes studied are given in Table 6 along with the target values.

For RDX, DNB, TNT and 2,4-DNT, the results from the two columns were not significantly different at the 95% confidence level and mean values were within 15% of the target values. For HMX and TNB, mean values for samples processed with methanol were significantly different from those processed with acetonitrile. In both cases the methanol-processed samples were closer to the target values than the acetonitrile-processed samples. A look at the chromatograms (Fig. 3) shows significant baseline disturbance in the region where HMX elutes when acetonitrile was used for sample dilution, but not when methanol was used. The large difference obtained for TNB was unexpected and the cause is unclear. Unusual results for TNB have been noted, however, for some soil extracts in acetonitrile (Jenkins et al. 1988). Results for samples diluted with methanol were much closer to the target values than were those where acetonitrile was used. Overall, use of methanol for dilution prior to filtration is preferable.

For the above comparison, we calculated results for each diluent using response factors obtained for standards prepared in a matched matrix. The question remains whether standards used to establish response factors for determinations on samples diluted with methanol must also be prepared in methanol. This is important, since soil extracts are generally analyzed in a 1:1 water/acetonitrile matrix against standards also prepared in water–acetonitrile.

To investigate this question, two separate combined-analyte stock standards were pre-

Table 6. Comparison of low level determinations (analyte concentration in µg/L) using either methanol or acetonitrile to dilute aqueous samples prior to filtration.

	HMX	RDX	TNB	DNB	TNT	2,4-DNT
Target value	62.4	70.4	63.8	64.8	66.2	64.6
Methanol	59.4*	64.9	51.0*	55.6	58.4	57.5
Acetonitrile	69.3*	62.7	37.6*	57.9	59.4	57.9

* Significantly different at the 95% confidence level using the Student's t test.

Water/Acetonitrile

HMX RDX TNB DNB TNT 2,4 DNT

Water/Methanol

HMX RDX TNB DNB TNT 2,4 DNT

0 2 4 6 8 10 12
Retention Time (min)
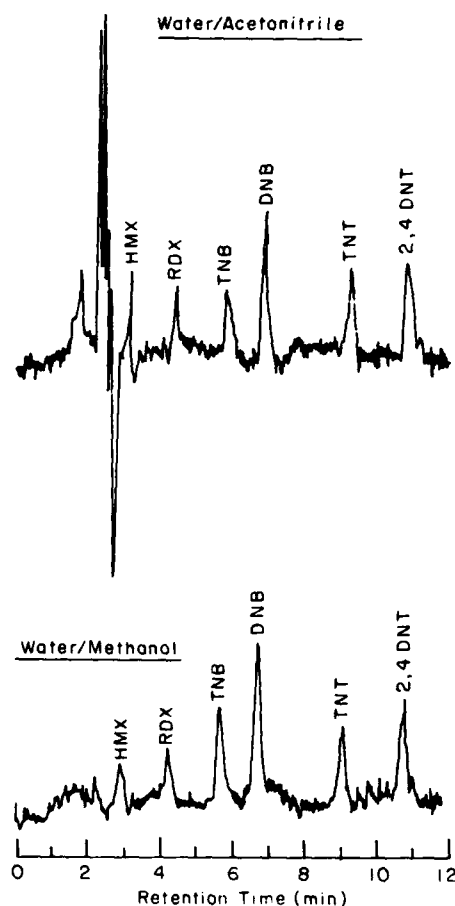
Figure 3. Comparison of chromatograms for samples diluted 1:1 with acetonitrile versus samples diluted 1:1 with methanol.

pared from the individual stock standards. One was diluted to volume with methanol and the other with acetonitrile. Each standard was further diluted 1:10 with the appropriate solvent and five 5.00-mL replicates of each were placed in individual 20-mL scintillation vials containing 5.00 mL of type 1 water. The ten individual standards were analyzed randomly and the results are shown in Table 7.

Clearly, mean responses for HMX, RDX and TNB differ considerably between the two solvents. Results for DNB, TNT and 2,4-DNT in the two solvents are much closer. The Relative Standard Deviations (RSD's) for the standards prepared in methanol are all lower than 1.0% and are all lower than the RSD's for the standards in acetonitrile. The RSD for TNB in particular (7.39%) is nearly ten times higher than that obtained in methanol (0.852%).

The differences observed in mean responses for standards prepared in different matrices were also observed for HMX and RDX in the ruggedness test conducted during the establishment of the standard water method (Jenkins et al. 1984). No clear-cut explanation was offered but the differences parallel those found here.

Overall, these results indicate that standards prepared for use with water samples that are diluted with methanol prior to filtration must also be prepared in water–methanol or a large bias will result. This is particularly true for HMX and RDX but it is also true to a lesser degree for TNB.

Table 7. Comparison of responses (peak height) for standards diluted to volume with either methanol or acetonitrile prior to final 1:1 dilution with water.

		HMX	RDX	TNB	DNB	TNT	2,4-DNT
Methanol	$\bar{x}$	113,499	137,501	246,841	335,154	197,669	241,404
	s	620	885	2102	2203	1097	1584
	s^2	3.84×10^5	7.84×10^5	4.42×10^6	4.86×10^6	1.20×10^6	2.51×10^6
	RSD*	0.547%	0.644%	0.852%	0.657%	0.555%	0.656%
Acetonitrile	$\bar{x}$	169,851	155,477	229,802	339,669	201,896	239,556
	s	4867	1253	16,989	3716	2427	1897
	s^2	2.37×10^7	1.57×10^6	2.89×10^8	1.38×10^7	5.89×10^6	3.60×10^6
	RSD	2.86%	0.806%	7.39%	1.09%	1.20%	0.792%

* Relative standard deviation.

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Comparison of determinations on real samples using procedures based on the LC-8 and the LC-18

We obtained three water samples from the Iowa Army Ammunition Plant to allow us to compare analytical results for the LC-8 based method using a mobile phase of 50:38:12 water/methanol/acetonitrile with those for the LC-18 based method using 50:50 water/methanol. These samples were obtained from a waste stream before treatment with activated carbon (sample 1), after treatment with one activated carbon column (sample 2) and after two activated carbon columns (sample 3). Samples 1 and 2 were diluted 1:10 with local groundwater prior to analysis, since preliminary results indicated extremely high concentrations of RDX and TNT. We found concentrations of analytes in sample 3 to be below the limits of determination on either column.

Twenty 5.00-mL aliquots of diluted Iowa sample 1 and sample 2 were placed in individual 20-mL glass scintillation vials. These replicates were randomly divided into two groups of 10 for each water sample. To one group, a 5.00-mL aliquot of methanol was added, then the solution was mixed thoroughly and filtered through a 0.5-μm Millex-SR filter. The other group was diluted with 5.00 mL of a mixed solvent made of 76% methanol and 24% acetonitrile (V/V) and processed in a similar manner. The first group was analyzed on an LC-18 column using a mobile phase consisting of 1:1 water/methanol. The second group was analyzed in a like manner on an LC-8 column with a mobile phase of 50:38:12 water/methanol/acetonitrile as described in AOAC (1986). We present the results of these determinations in Appendix A, Table A1, and show a summary in Table 8.

Statistical analysis indicates that only for TNB in Iowa sample 1 were mean values on the two columns different at the 95% confidence level. For all other determinations, means were not significantly different, even where relative standard deviations averaged between 1 and 2%, demonstrating the ability to detect very small differences. The difference observed for TNB in sample 1 is only 7% at a concentration of about 70 μg/L. The reason for the observed difference for TNB here, but not in sample 2, is unclear. Unusual results for TNB have been observed in several instances. We also observed a rather large difference for TNB when comparing the results for low level standards on LC-18 using methanol or acetonitrile, or both, as the organic diluent prior to filtration. In both cases the found concentration of TNB is lower with acetonitrile present than where methanol is the only organic solvent present.

Overall, determinations using the LC-18 column with a mobile phase composed of 1:1 methanol/water give equivalent results to the standardized procedure (AOAC 1986) using the LC-8 separation for the two samples from the Iowa AAP.

Linearity testing

To define whether detector response is linear with concentration of standard, two sets of calibration standards were independently prepared for each of two stock solutions as de-

Table 8. Comparison of analytical results (concentration in μg/L) for aqueous samples from Iowa AAP using either LC-8 or LC-18 columns.

Sample		HMX LC-8	HMX LC-18	RDX LC-8	RDX LC-18	TNB LC-8	TNB LC-18	TNT LC-8	TNT LC-18
Iowa 1	$\bar{x}$	<d	<d	415	431	69.1*	74.3*	10,560	10,636
	s	—	—	13.6	22.9	5.3	3.2	146	59
	RSD	—	—	3.3%	5.3%	7.7%	4.4%	1.4%	0.6%
Iowa 2	$\bar{x}$	188	184	2121	2117	27.2	27.3	1762	1746
	s	19.3	8.4	40	29	4.4	3.8	22	27
	RSD	10.3%	4.6%	1.9%	1.4%	16.0%	13.8%	1.2%	1.5%

* Means are significantly different at the 95% confidence level using the Student's t test.

scribed in Tables 1 and 2. We analyzed these standards as usual, using the LC-18 column with a 1:1 water/methanol eluent. Peak heights for each of the tested analytes were obtained from a digital integrator. Results are presented in Table A2.

For each analyte, a linear model with intercept was fitted to the data using standard linear regression. The residual sums of squares were tested for significance using a lack-of-fit test at the 95% confidence level as described in USATHAMA (1985). Except for tetryl (Table A2e), the F-ratios for standards over the entire concentration range indicated that a linear model adequately described each data set. Next, a linear model without intercept was fitted to each data set. Using this model, we compared the residuals to those we calculated using the model with an intercept and an F-ratio obtained as described in USATHAMA (1985). In all cases the F-ratios at the 95% confidence level indicated that a linear model through the origin adequately described the data. Thus, for daily calibration, a replicated single point calibration is sufficient. The sensitivity and proven ranges of linear response for each analyte are presented in Table 9.

For tetryl, the F-ratio calculated over the entire range (Table A2e) indicated that the data set was not adequately described by a linear model. When we dropped the highest standard, however, the lack-of-fit F-ratio for the concentration range up to 230 µg/L (Table A2f) was not significant, indicating that

within this reduced range the linear model was an adequate description of the data. Within this range, a linear model with zero intercept was also found to be adequate for describing the calibration relationship between concentrations and detector response. Thus, for daily calibration a replicated single standard within the linear ranges described is adequate.

Method reporting limits

We conducted method reporting limit tests as described in USATHAMA (1985) using the general method developed by Hubaux and Vos (1970). A target reporting limit was estimated using signal-to-noise measurements for each analyte and samples were prepared at 0, 0.5, 1, 2, 5 and 10 times these estimated levels. Determinations at each level were made in duplicate in random order on each of four days. Results are presented in Table A3.

We obtained means and variances of found concentrations for each spike level. Bartlett's test (USATHAMA 1985) was used to establish the range of homogeneous variance for each analyte (Table 10). Then, we regressed found concentrations against target concentrations using the data for which the variances were homogeneous. Overall analyte recovery or accuracy was estimated using the slope of the best fit regression line. These values are presented in Table 11. Confidence limits were obtained about these regression lines at the 90% level according to USATHAMA (1985). The reporting limit was obtained from the value of the target concentration corresponding to the point on the lower confidence limit curve where the value of the found concentration equals the value on the upper confidence limit curve at target concentration = 0. An example is given for DNB in Figure 4. Reporting limit values obtained in this way are presented in Table 11.

The random error variances at each tested concentration were used to define analytical precision for each analyte. Since variances were homogeneous over the entire ranges examined for all analytes tested except HMX, analytical precision was estimated from the pooled standard deviations over each range. Values are shown in Table 11.

The accuracy estimates range from 93.3 for TNB to 100.5 for 2,4-DNT, with an average value of 99% for the 13 analytes tested.

Table 9. Results of linearity testing.

Analyte	Sensitivity (absorbance per µg/L)	Linear range (µg/L)
HMX	2.96×10^{-6}	40.5 – 1013
RDX	3.55×10^{-6}	10.0 – 1003
TNB	6.67×10^{-6}	5.0 – 505
DNB	8.77×10^{-6}	2.6 – 514
Tetryl	4.90×10^{-6}	4.6 – 230
TNT	5.22×10^{-6}	2.3 – 457
2,4-DNT	6.24×10^{-6}	2.5 – 405
NB	9.53×10^{-6}	6.6 – 656
2,6-DNT	5.49×10^{-6}	10.0 – 1002
2-Am-DNT	1.02×10^{-5}	6.3 – 630
o-NT	——	——
p-NT	——	——
m-NT	——	——

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Table 10. Variance analysis at measured concentrations for reporting limit test.

Analyte	Target concentration (μg/L)	Found Mean (μg/L)	Found Variance	Bartlett's test (X^2)*
HMX	10.1	14.0	4.3	
	20.3	24.3	22.6	
	40.5	42.8	22.6	
	101.3	102.9	53.1	
	202.6	201.2	11.3	1.36
RDX	10.0	12.3	2.3	
	20.1	25.7	13.8	
	40.1	44.5	31.7	
	100.3	101.6	12.4	
	200.6	202.5	50.9	1.53
TNB	2.0	4.0	4.5	
	4.0	6.0	5.4	
	8.1	10.0	6.2	
	20.2	19.6	2.0	
	40.4	40.2	3.5	1.07
DNB	2.1	2.9	0.2	
	4.1	5.4	1.6	
	8.2	9.3	2.8	
	20.5	21.2	1.9	
	41.1	40.7	5.7	1.62
NB	3.2	4.2	0.4	
	6.4	8.8	3.2	
	12.8	15.0	5.8	
	32.1	33.3	5.8	
	64.1	65.0	3.2	1.38
TNT	1.8	3.1	0.2	
	3.7	6.9	5.8	
	7.3	9.7	5.0	
	18.3	19.6	5.2	
	36.5	37.9	3.7	1.61
2,6-DNT	2.0	4.6	2.8	
	4.0	9.0	11.3	
	8.0	11.3	4.8	
	20.0	22.4	9.1	
	40.1	41.6	4.6	1.13
2,4-DNT	2.0	4.4	1.8	
	4.0	6.6	5.7	
	8.1	9.7	2.2	
	20.2	21.7	1.0	
	40.4	42.1	3.7	1.18
o-NT	10.7	13.3	3.8	
	21.5	25.9	4.1	
	42.9	47.7	2.1	
	107.3	108.9	33.8	
	214.5	217.7	30.6	1.85

Table 10 (cont'd). Variance analysis at measured concentrations for reporting limit test.

Analyte	Target concentration ($\mu g/L$)	Found Mean ($\mu g/L$)	Found Variance	Bartlett's test (X^2)*
p-NT	12.8	16.6	4.7	
	25.6	31.6	8.6	
	51.1	56.8	7.4	
	127.8	132.4	53.8	
	255.5	259.5	37.3	1.56
m-NT	11.1	15.0	4.2	
	22.1	26.8	1.7	
	44.2	47.9	9.0	
	110.5	114.2	61.7	
	221.0	224.0	28.4	2.04
Tetryl	9.2	16.4	21.7	
	18.4	25.3	64.9	
	36.8	43.1	122.3	
	92.0	93.4	616.0	2.36
	184.0	183.3	1103.9	
2,4,5-TNT	2.1	7.6	13.3	
	4.3	8.0	12.0	
	8.5	10.7	10.4	
	21.3	24.0	38.5	
	42.5	43.9	56.6	1.28

* Critical values for X^2 = 9.49 at the 95% confidence level for five concentration ranges.

Table 11. Results of reporting limit tests.

Analyte	Certified reporting limit* ($\mu g/L$) LC-18	Certified reporting limit* ($\mu g/L$) LC-8†	LC-18 method Accuracy** (%)	LC-18 method Precision†† ($\mu g/L$)	MDL***
HMX	13.0	26	98.1	4.7	13.0
RDX	14.0	22	99.4	4.7	12.0
TNB	7.3	—	95.3	2.1	5.0
DNB	4.0	—	97.7	1.6	2.4
Tetryl	44.0	—	96.7	19.3	26.0
TNT	6.9	14	99.8	2.0	2.6
2.4-DNT	5.7	10	100.5	1.7	15.7
2,6-DNT	9.4	—	98.1	2.6	5.1
NB	6.4	—	99.8	1.9	3.1
2,4,5-TNT	14.0	—	96.9	5.1	31.0
o-NT	12.0	—	100.3	3.9	12.0
m-NT	7.9	—	100.2	4.6	—
p-NT	8.5	—	100.4	4.7	30.0
2-Am-DNT	—	—	—	—	18.0

* According to method developed by Hubaux and Vos (1970).
† Jenkins et al. (1984).
** Calculated from slope of regression of target vs found concentrations.
†† Pooled standard deviation of found values in range of homogeneous variance during CRL test.
*** According to EPA protocol.
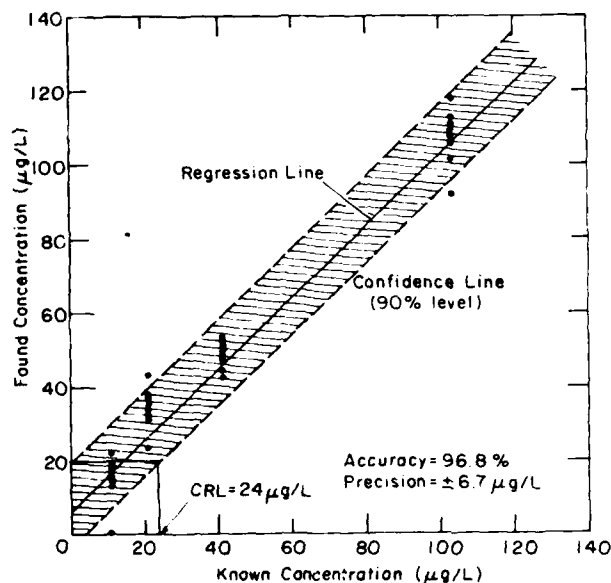
Figure 4. Regression analysis procedure used to calculate the certified reporting limit using the 90% confidence lines.

Precision values range from ± 1.6 μg/L for DNB to ± 19.3 μg/L for tetryl. At the highest concentrations tested, these absolute standard deviations convert to RSD values ranging from 3.9% for DNB to 21% for tetryl.

Instability of tetryl in methanol–water matrix

One of the goals of this work was to develop a method that could be used to determine tetryl in addition to HMX, RDX, TNB, DNB, TNT and the 2,4- and 2,6-isomers of DNT. The LC-18 column provides excellent separation of tetryl from the other primary analytes and the most likely interferences. Linearity tests showed that, for samples processed quickly, tetryl gave a linear response with respect to concentrations. In our certified reporting limit tests, however, samples were run over an extended period and we observed that peak heights for tetryl in the standard decreased significantly with time. As shown in the chromatogram in Figure 5, over a 24-hour period the peak heights for the other analytes remain constant but the peak height for tetryl decreases by two-thirds. As tetryl declines, a peak eluting about 0.8 min after tetryl in-

creases, apparently a result of a decomposition product. A second peak also becomes visible as a shoulder on TNT, but does not interfere with TNT if peak height measurements are used.

We also examined the stability of tetryl in a water–acetonitrile matrix and found it to remain stable over a 48-hour period. Thus, if tetryl is an important analyte for a given study, we recommend dilution with acetonitrile prior to filtration. In general, however, this is not recommended, since much better analyte recovery and precision were obtained for a number of other analytes (HMX, RDX, TNB) when the water was diluted with methanol rather than acetonitrile.

Comparison of LC-18 protocol to LC-8 protocol

The step-by-step procedure in USATHAMA format is presented in Appendix B. This method differs from the Standard Method (AOAC 1986) in two ways. First, aqueous samples are diluted 1:1 with methanol prior to filtration rather than being diluted 1:1 with a solution composed of 76% methanol and 24% acetonitrile. Second, the separation is achieved using an LC-18 RP-HPLC column
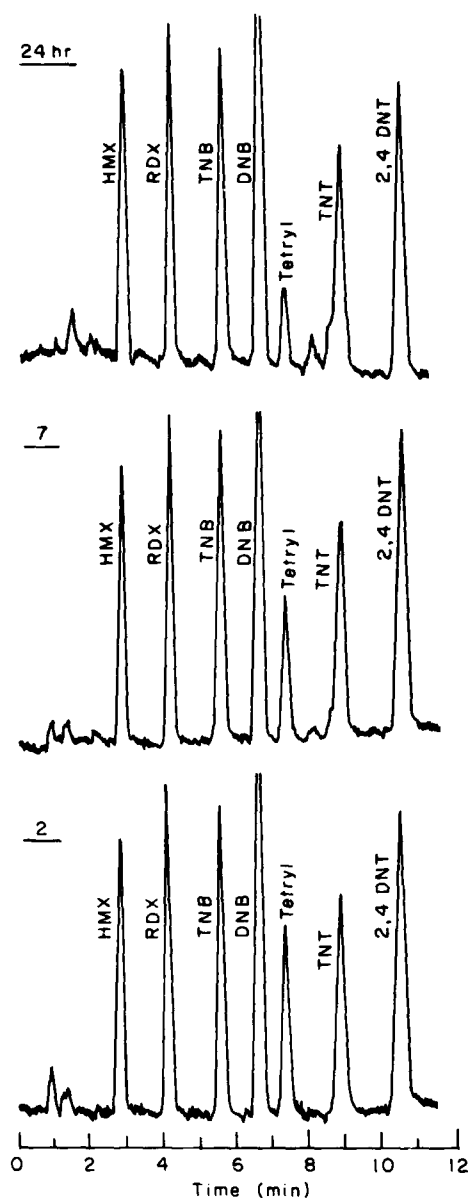
Figure 5. Comparison of chromatograms of analytes after set time intervals (2, 7 and 24 hours).

rather than an LC-8 column, allowing analysis of water and soil extracts on the same column and eluent combination.

The change to pure methanol from methanol–acetonitrile is advantageous in all respects. It requires less manipulation, is less expensive to use and the solvent is less toxic. The presence of acetonitrile has also been shown to be troublesome when determining TNB.

Use of LC-18 with a methanol–water eluent rather than LC-8 with a ternary eluent is also an improvement. The LC-18 column gives much better analyte resolution in about the same overall run time. It separates tetryl from TNT, a separation we could not achieve on LC-8 using isocratic conditions. Certified reporting limits for the four analytes, where a comparison between the LC-18 and earlier LC-8 based methods was possible, indicate lower levels were achieved using the LC-18 method. While part of this improvement is undoubtedly attributable to the use of a new variable-wavelength detector with lower noise levels, the new LC-18 based method appears to be at least as good in this regard as the older LC-8 based procedure. Thus, for many of the analytes tested, CRL's under 10 $\mu g/L$ are attainable for water analysis with no extraction or preconcentration required.

SUMMARY AND CONCLUSIONS

We developed a protocol for determining a number of nitroaromatics and nitramine explosives in water using an isocratic RP-HPLC method on an LC-18 column. The method calls for dilution of an aqueous sample 1:1 with methanol, filtration through a 0.5-μm Millex-SR filter, separation on an LC-18 column using a 1:1 water/methanol eluent and determination by UV-254 nm. The method was shown to average about a 99% recovery for the following analytes: HMX, RDX, TNB, DNB, NB, TNT, 2,4-DNT, 2,6-DNT, 2,4,5-TNT, o-NT, m-NT and p-NT. Certified reporting limits for these 12 analytes ranged from 4–15 $\mu g/L$, with analytical precision ranging from 1.6–5.1 $\mu g/L$.

The method did not work as well for tetryl, even though excellent separation was achieved on the LC-18 column. This was caused by slow decomposition of tetryl in the water–methanol matrix. Dilution of the aqueous sample with acetonitrile rather than methanol eliminates the problem with tetryl decomposition, but results in poorer method performance for HMX, RDX and TNB.

Since we have previously developed a protocol for determination of explosive residues in soil using RP-HPLC on LC-18, acceptance

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of this water protocol allows water and soil extract determinations on the same column and eluent combination. However, standards must be prepared in the same matrix as the samples to be determined. For water, a standard prepared in water–methanol must be used, while for soil extracts, a standard in water–acetonitrile is necessary. This is not a prohibitive requirement, however, since in both cases response is linear and a zero intercept model adequately describes the relationship between concentration and response, allowing calibration with a single standard.

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APPENDIX A: DATA

Table A1. Result (concentration in μg/L) of analysis of Iowa AAP water samples.

Sample	Replicate	HMX LC-8	HMX LC-18	RDX LC-8	RDX LC-18	TNB LC-8	TNB LC-18	TNT LC-8	TNT LC-18
Iowa 1	1	<d	<d	391	373	61.0	71.4	10519	10666
	2	"	"	428	444	77.9	73.1	10552	10609
	3	"	"	417	427	65.6	71 7	10638	10656
	4	"	"	422	448	71.9	72.4	10616	10604
	5	"	"	416	431	75.2	77.4	10633	10571
	6	"	"	419	453	67.5	76.7	10690	10692
	7	"	"	426	420	70.6	68.7	10712	10696
	8	"	"	430	432	72.2	78.2	10573	10609
	9	"	"	409	433	65.8	77.6	10207	10532
	10	"	"	393	447	63.5	75.4	10457	10715
Iowa 2	1	217	186	2179	2160	a	25.0	1754	1751
	2	193	181	2165	2106	33.7	24.3	1780	1745
	3	172	185	2139	2101	25.1	a	1788	1741
	4	a	a	2061	2103	28.3	23.9	1786	1717
	5	198	a	2103	2065	26.8	34.3	1737	1698
	6	231	171	2117	2165	a	25.0	1770	1793
	7	193	197	2162	2129	a	24.8	1770	1759
	8	165	a	2098	2112	22.0	32.3	1761	1756
	9	179	a	2118	2124	a	a	1753	1770
	10	165	182	2068	2104	a	30.8	1720	1731

a - Peak visible but no value output by integrator.

Table A2. Results of linearity testing.

a. HMX.

Standard concentration (μg/L)	Peak height A	Peak height B
5.1	0	0
10.1	0	0
20.3	1016	856
50.7	1276	1194
101.3	2554	2526
202.6	5103	4935
506.5	12293	12289
1013	21263	23884

Lack-of-fit test

Peak height = 211.0 + 22.47 [conc.]
Lack-of-fit F-ratio calculated = 0.85
Critical F-ratio (95% level) = 3.50
Conclusion: linear model accepted.

Zero intercept test

Peak height = 22.77 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 1.16
Critical F-ratio (95% level) = 4.60
Conclusion: intercept nonsignificant.

b. RDX.

Standard concentration (μg/L)	Peak height A	Peak height B
5.0	0	0
10.0	427	513
20.1	1036	898
50.2	1734	1447
100.3	3116	3298
200.6	5805	5953
501.5	14813	14178
1003	28045	24162

Lack-of-fit test

Peak height = 401.3 + 26.16 [conc.]
Lack-of-fit F-ratio calculated = 0.56
Critical F-ratio (95% level) = 3.58
Conclusion: linear model accepted.

Zero intercept test

Peak height = 26.74 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 2.14
Critical F-ratio (95% level) = 4.60
Conclusion: intercept nonsignificant.

Table A2 (cont'd). Results of linearity testing.

c. TNB

Standard concentration (µg/L)	Peak height A	Peak height B
2.5	0	0
5.0	564	647
10.1	968	1089
25.2	1539	1515
50.5	2778	2864
100.9	5652	5431
252.3	13780	14148
504.5	23256	26837

Lack-of-fit test

Peak height = 386.9 + 49.88 [conc.]
Lack-of-fit F-ratio calculated = 0.64
Critical F-ratio (95% level) = 3.58
Conclusion: linear model accepted.

Zero intercept test

Peak height = 50.99 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 2.29
Critical F-ratio (95% level) = 4.60
Conclusion: intercept nonsignificant.

d. DNB.

Standard concentration (µg/L)	Peak height A	Peak height B
2.6	568	0
5.1	621	673
10.3	1303	1231
25.7	1962	2140
51.4	3680	3556
102.7	7712	7406
256.8	18317	19067
513.5	30334	35538

Lack-of-fit test

Peak height = 565.2 + 64.60 [conc.]
Lack-of-fit F-ratio calculated = 0.67
Critical F-ratio (95% level) = 3.50
Conclusion: linear model accepted.

Zero intercept test

Peak height = 66.20 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 2.24
Critical F-ratio (95% level) = 4.00
Conclusion: Intercept nonsignificant.

e. Tetryl over full range tested.

Standard concentration (µg/L)	Peak height A	Peak height B
2.3	0	0
4.6	420	579
9.2	745	830
23.0	1027	1215
46.0	1487	1414
92.0	3871	3168
230.0	8402	8736
460.0	13632	11789

Lack-of-fit test

Peak height = 518.2 + 28.27 [conc.]
Lack-of-fit F-ratio calculated = 4.87
Critical F-ratio (95% level) = 3.58
Conclusion: data not linear.

f. Tetryl in range up to 230 µg/L.

Standard concentration (µg/L)	Peak height A	Peak height B
2.3	0	0
4.6	420	579
9.2	745	830
23.0	1027	1215
46.0	1487	1414
92.0	3871	3168
230.0	8402	8736

Lack-of-fit test

Peak Height = 165.4 + 36.33 [conc.]
Lack-of-fit F-ratio calculated = 2.79
Critical F-ratio (95% level) = 3.97
Conclusion: linear model accepted.

Zero intercept test

Peak height = 37.38 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 2.85
Critical F-ratio (95% level) = 4.75
Conclusion: intercept nonsignificant.

g. TNT.

Standard concentration (µg/L)	Peak height A	Peak height B
2.3	375	0
4.6	511	503
9.1	1071	697
22.8	886	1548
45.7	2024	2064
91.3	4440	3861
228.3	9712	10359
456.5	15969	19096

Lack-of-fit test

Peak height = 439.4 + 38.40 [conc.]
Lack-of-fit F-ratio calculated = 0.52
Critical F-ratio (95% level) = 3.58
Conclusion: linear model accepted.

Zero intercept test

Peak Height = 39.79 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 3.65
Critical F-ratio (95% level) = 4.60
Conclusion: intercept nonsignificant.

h. 2,4-DNT.

Standard concentration (µg/L)	Peak height A	Peak height B
2.5	463	0
5.0	540	0
10.1	572	1029
25.2	1542	1354
50.5	2735	2526
100.9	5958	5120
252.3	13100	13536
504.5	21341	24973

Lack-of-fit test

Peak height = 406.8 + 46.42 [conc.]
Lack-of-fit F-ratio calculated = 0.84
Critical F-ratio (95% level) = 3.50
Conclusion: linear model accepted.

Zero intercept test

Peak height = 47.58 [conc.]
Zero intercept hypothesis F-ratio
  calculated = 2.01
Critical F-ratio (95% level) = 4.60
Conclusion: intercept nonsignificant.

Table A2 (cont'd).

i. NB.

Standard concentration (μg/L)	Peak height A	Peak height B
6.6	0	0
13.1	0	2417
26.2	4675	2636
65.6	6039	5209
131.1	9862	11954
262.2	19294	18679
655.5	48707	45689

Lack-of-fit test

Peak height = 749.3 + 70.96 [conc.]
Lack-of-fit F-ratio calculated = 0.85
Critical F-ratio (95% level) = 3.97
Conclusion: linear model accepted.

Zero intercept test

Peak height = 72.63 [conc.]
Zero intercept hypothesis F-ratio calculated = 3.02
Critical F-ratio (95% level) = 4.75
Conclusion: intercept nonsignificant.

j. 2,6-DNT.

Standard concentration (μg/L)	Peak height A	Peak height B
10.0	0	0
20.0	0	2368
40.1	101	2742
100.2	4978	4793
200.4	8681	10828
400.8	17447	17467
1002.0	43017	39559

Lack-of-fit test

Peak height = 626.3 + 40.94 [conc.]
Lack-of-fit F-ratio calculated = 0.58
Critical F-ratio (95% level) = 3.97
Conclusion: linear model accepted.

Zero intercept test

Peak height = 41.85 [conc.]
Zero intercept hypothesis F-ratio calculated = 2.60
Critical F-ratio (95% level) = 4.75
Conclusion: intercept nonsignificant.

k. 2-Am-DNT.

Standard concentration (μg/L)	Peak height A	Peak height B
6.3	0	0
12.6	0	2092
25.2	3150	3940
63.0	5214	5442
126.0	10185	12123
252.0	19860	19725
630.0	50676	46301

Lack-of-fit test

Peak height = 614.5 + 76.27 [conc.]
Lack-of-fit F-ratio calculated = 0.18
Critical F-ratio (95% level) = 3.97
Conclusion: linear model accepted.

Zero intercept test

Peak height = 77.70 [conc.]
Zero intercept hypothesis F-ratio calculated = 1.92
Critical F-ratio (95% level) = 4.75
Conclusion: intercept nonsignificant.

Table A3. Results of reporting limit test.

Spike level	Spiked concentration (μg/L)	Found concentration (μg/L) Day 1	Day 2	Day 3	Day 4
a. HMX					
0	0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	10.1	16.5	14.2	13.1	13.1
		17.1	13.0	13.9	12.1
X	26.3	30.5	23.3	22.4	21.6
		32.9	20.3	22.9	20.5
2X	40.5	49.5	40.1	41.1	38.1
		50.7	40.0	43.5	39.5
5X	101.3	116.9	98.4	105.4	99.0
		101.3	105.9	104.6	92.0
10X	202.6	200.0	207.6	203.3	201.2
		198.4	202.5	196.6	199.9
b. RDX					
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	10.0	11.3	10.8	11.1	12.2
		10.94	13.3	14.7	14.1
X	20.0	31.5	23.6	23.3	24.1
		31.4	26.2	22.9	22.7
2X	40.1	53.9	42.9	42.8	40.8
		52.6	38.4	41.3	43.1
5X	100.3	102.3	95.1	104.1	100.1
		102.0	106.7	103.4	99.3
10X	200.6	207.1	207.3	186.8	208.5
		201.1	200.4	201.4	207.2

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Table A3 (cont'd). Results of reporting limit test.

Spike level	Spiked concentration (μg/L)	Found concentration (μg/L)			
		Day 1	Day 2	Day 3	Day 4
c. TNB					
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	2.02	7.3	2.4	4.2	2.5
		7.4	2.5	3.2	2.7
X	4.04	8.6	4.9	5.0	4.6
		10.6	5.8	4.0	4.6
2X	8.07	14.3	9.6	9.7	8.1
		13.4	8.1	8.0	8.7
5X	20.2	18.0	18.5	18.4	20.1
		19.8	22.5	20.2	19.3
10X	40.4	40.9	40.0	39.4	40.5
		39.3	44.4	38.3	39.1
d. DNB					
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	2.1	2.5	2.8	3.6	2.8
		2.3	3.0	3.0	2.8
X	4.1	8.2	5.8	4.4	4.6
		5.9	4.5	4.7	4.7
2X	8.2	12.0	8.0	8.8	8.7
		11.8	8.0	8.0	9.0
5X	20.2	20.0	21.9	20.2	21.6
		20.7	24.2	20.4	20.3
10X	40.4	36.1	42.5	38.2	42.3
	41.2	42.7	40.7	42.3	
e. NB					
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	3.2	4.1	4.1	5.7	4.0
		3.6	4.2	4.0	3.9
X	6.4	12.6	8.2	7.3	7.5
		10.2	8.0	9.1	7.7
2X	12.8	18.4	13.0	13.6	13.9
		19.0	12.6	15.4	14.3
5X	32.1	31.3	32.8	32.2	32.7
		33.4	39.1	32.4	32.4
10X	64.1	63.5	66.0	62.0	65.4
		66.0	67.6	64.0	65.0

Table A3 (cont'd).

Spike level	Spiked concentration (µg/L)	Found concentration (µg/L)			
		Day 1	Day 2	Day 3	Day 4

f. 2,4,6-TNT

0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	1.83	3.3	2.8	3.0	2.9
		2.8	2.8	4.0	2.8
X	3.65	10.5	7.5	5.0	4.1
		10.3	5.5	6.6	5.4
2X	7.30	12.7	7.8	9.6	8.2
		13.2	7.1	9.8	8.7
5X	18.26	17.6	17.8	19.1	19.1
		19.3	24.7	20.9	18.5
10X	36.52	35.3	38.5	35.2	37.2
		38.3	41.0	39.1	38.4

g. 2,6-DNT

0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	2.00	6.0	3.2	6.2	3.3
		5.5	2.6	6.7	3.1
X	4.01	13.1	10.7	6.5	5.6
		14.3	7.9	8.0	5.7
2X	8.02	15.1	8.6	10.6	9.4
		13.7	10.7	12.0	10.2
5X	20.04	23.0	20.6	20.7	21.7
		21.6	29.1	18.7	23.0
10X	40.08	39.6	43.0	40.6	41.5
		42.0	46.1	40.4	39.7

h. 2,4-DNT

0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	2.02	5.0	2.9	5.7	3.0
		5.4	5.8	4.4	2.7
X	4.04	5.2	4.4	5.6	8.6
		4.9	9.7	10.1	4.6
2X	8.07	12.1	8.6	11.5	7.7
		10.2	9.6	9.8	8.6
5X	20.18	21.0	21.4	23.6	21.1
		22.0	20.9	20.7	22.7
10X	40.36	41.8	41.0	42.7	45.2
		39.7	39.9	42.4	44.1

Table A3 (cont'd). Results of reporting limit test.

Spike level	Spiked concentration (µg/L)	Found concentration (µg/L)			
		Day 1	Day 2	Day 3	Day 4
i. o-NT					
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	10.7	13.3	13.0	15.0	9.6
		13.5	15.4	15.0	11.6
X	21.5	27.0	25.9	25.7	25.5
		22.6	24.5	29.7	26.3
2X	42.9	48.4	45.6	46.9	48.9
		48.9	45.8	49.2	48.0
5X	107.3	106.5	106.3	108.7	109.7
		110.0	121.2	108.1	100.5
10X	214.5	208.6	222.8	212.6	220.7
		221.3	221.4	212.6	221.9
j. p-NT					
0	0.0	10.0	0.0	0.0	36.6
		0.0	0.0	0.0	0.0
0.5X	12.78	17.4	14.5	18.3	15.3
		17.5	15.4	20.6	14.3
X	25.55	33.5	35.0	35.4	26.9
		28.9	31.2	31.6	30.5
2X	51.50	59.0	51.7	59.8	56.4
		59.1	56.2	57.6	54.5
5X	127.75	127.5	129.5	129.9	135.4
		134.2	148.4	129.9	124.6
10X	255.50	252.4	263.9	251.7	264.9
		263.1	262.4	252.6	265.2
k. m-NT					
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	11.05	14.2	13.1	17.1	13.4
		14.2	16.6	18.4	13.2
X	22.10	25.7	28.5	28.0	25.8
		24.9	28.1	26.8	27.0
2X	44.20	48.0	42.9	52.7	48.7
		47.6	44.9	50.2	48.1
5X	110.50	108.5	111.3	112.7	114.9
		112.5	132.7	113.3	107.7
10X	221.00	216.4	225.7	218.2	230.2
		228.6	226.9	218.9	227.0

Table A3 (cont'd).

Spike level	Spiked concentration (μg/L)	Found concentration (μg/L)			
		Day 1	Day 2	Day 3	Day 4

1. tetryl

Spike level	Spiked concentration (μg/L)	Day 1	Day 2	Day 3	Day 4
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	9.20	21.3	16.8	19.2	12.4
		23.4	15.5	11.1	11.3
X	18.40	23.6	18.2	24.4	41.6
		25.4	24.4	30.2	14.9
2X	36.80	50.8	34.5	46.7	54.5
		46.5	27.3	29.6	52.1
5X	92.00	102.0	114.5	70.1	114.5
		54.8	117.1	68.9	105.6
10X	184.00	249.1	188.6	172.5	166.7
		193.6	196.0	134.2	166.0

m. 2,4,5-TNT

Spike level	Spiked concentration (μg/L)	Day 1	Day 2	Day 3	Day 4
0	0.0	0.0	0.0	0.0	0.0
		0.0	0.0	0.0	0.0
0.5X	2.13	12.7	6.5	8.5	3.6
		12.8	5.8	8.1	3.2
X	4.25	4.6	5.2	7.1	10.7
		6.0	9.9	14.8	6.1
2X	8.50	17.1	8.9	13.8	7.5
		10.7	9.4	10.4	8.1
5X	21.26	21.6	25.0	37.9	20.2
		22.1	17.1	25.3	22.7
10X	42.52	51.1	43.3	40.9	49.5
		33.9	33.3	53.1	46.3

Reversed-Phase HPLC Method for the Determination of
Nitroaromatics and Nitramines in Water

I. **Summary**

A. **Analytes**: The following analytes can be determined using this
analytical method: HMX, RDX, 135TNB, 13DNB, 246TNT, 24DNT, 26DNT, NB, 2NT,
3NT, 4NT and TETRYL.

B. **Matrix**: This method is suitable for the determination of nitroaro-
matics and nitramines in a water matrix.

C. **General Method**: This method involves diluting the water sample
1:1 with methanol, mixing thoroughly, filtering and determining by reversed-
phase HPLC on an LC-18 column, using an eluent of 1:1 (V/V) water-methanol,
and UV detection at 254 nm.

II. **Application**

A. **Tested Concentration Range**: This method was found to be linear
over the following concentration ranges: HMX (10-203 μg/L) RDX (10-201
μg/L), 135TNB (2-40 μg/L), 13DNB (2-40 μg/L), 246TNT (2-36 μg/L), 24DNT
(2-40 μg/L), 26DNT (2-40.1 μg/L), NB (3-64 μg/L), 2NT (11-214 μg/L), 3NT
(11-221 μg/L), 4NT (13-256 μg/L), and TETRYL (9-184 μg/L).

B. **Sensitivity**: The response of the UV detector at 254 nm for the
various analytes is presented below.

Analyte	Sensitivity*
HMX	9.5×10^{-5}
RDX	8.0×10^{-5}
135TNB	7.2×10^{-5}
13DNB	4.8×110^{-5}
NB	4.4×10^{-5}
246TNT	4.3×10^{-5}
26DNT	3.4×10^{5}
24DNT	3.9×10^{-5}
2NT	3.5×10^{-5}
3NT	2.1×10^{-5}
4NT	2.0×10^{-5}
TETRYL	2.6×10^{-4}

* Absorbance units at the
Certified Reporting Limit.

C. <u>Reporting Limits</u>: Certified Reporting Limits (CRL) for the following analytes were determined over a 4-day period using the method of Hubaux and Vos as described in the USATHAMA Installation Restoration Program Quality Assurance Program (1987). CRL values for the various analytes are given below.

Analyte	Certified Reporting Limit (μg/L)
HMX	13
RDX	14
135TNB	7.3
13DNB	4.0
NB	6.4
246TNT	6.9
26DNT	9.4
24DNT	5.7
2NT	12
3NT	7.9
4NT	8.5
TETRYL	44

D. **Interferences**: Either 24DNT or 26DNT can be determined using this protocol, however, their retention times differ by only 0.2 min. Thus a large amount of either would preclude the low level determination of the other. The method was also qualitatively tested for a number of potential interferences. Retention times for certified analytes and potential interferences on LC-18 and LC-CN are presented in Table 1. Use of LC-CN for second column confirmation has proven satisfactory.

Table 1. Retention times and capacity factors for primary analytes and potential interferences on LC-18 and LC-CN columns eluted with 1:1 V\V water-methanol at 1.5 mL/min.

Substance	Retention Time (min)		Capacity Factor (k)*	
	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
135TNB	5.11	4.05	2.12	0.71
13DNB	6.16	4.18	2.76	0.76
246TNT	8.42	5.00	4.13	1.11
24DNT	10.05	4.87	5.13	1.05
TETRYL	6.93	7.36	3.23	2.11
NG	7.74	6.00	3.72	1.53
NB	7.23	3.81	3.41	0.61
3NT	14.23	4.45	7.68	0.88
4NT	13.26	4.41	7.09	0.86
2NT	12.26	4.37	6.48	0.84
2ADNT	9.12	5.65	4.56	1.38
4ADNT	8.88	5.10	4.41	1.15
SEX	2.40	5.07	0.46	1.14
TAX	2.78	3.70	0.70	0.56
245TNT	8.44	5.89	4.15	1.49
24DANT	3.16	4.20	0.93	0.77
26DANT	2.39	3.70	0.46	0.56
26DNT	9.82	4.61	4.99	0.95
Benzene	11.22	3.48	5.84	0.47
Toluene	23.0	3.93	13.02	0.66

* Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and 2.00 min on LC-CN.

E. **Analysis Rate**: Approximately 25 samples and working standards can be prepared and analyzed in an 8-hour day provided that stock solutions are already prepared and that the instrumentation is equipped with an auto sampler.

F. **Safety Information**: The normal safety precautions appropriate to use of flammable organic solvents should be employed.

III. **Apparatus and Chemicals**

    A. **Glassware/hardware**:

        1) Filters: 0.5-μm Millex-SR, disposable (1/sample).

        2) Pippettes: 50 mL (1), 10 mL (2), 5 mL (20), 4 mL (1), 2 mL (2), 1 mL (8) volumetric, glass.

        3) Volumetric flask: 100 mL (14), 250 mL (6), 200 mL (6), 50 mL (2).

        4) Scintillation vials: 20 mL, glass (2/sample).

        5) Auto sampler vials: glass (Septa Teflon faced) (1/sample).

        6) Disposable springs: Plastipak, 10 mL (1/sample).

    B. **Instrumentation**:

        1) HPLC Perkin Elmer Series 3 or Spectra-Physics SP8810 pump (or equivalent), an injector equipped with a 100-μL injection loop and a Spectra-Physics SP8490 UV detector set at λ = 254 nm (or equivalent fixed UV-254 or variable set at 254 nm). Both RP-HPLC columns are eluted at 1.5 mL/min with a 1:1 V/V methanol-water eluent.

        2) Strip chart recorder (Linear 500 or equivalent).

        3) Digital Integrator (HP3393A or equivalent).

        4) Autosampler (optional) (Dynatech Model LC-241 or equivalent).

        5) LC-18 (Supelco) RP-HPLC column 25-cm x 4.6-mm (5 μm).

        6) LC-CN (Supelco) RP-HPLC column 25-cm x 4.6-mm (5 μm).

C. Analytes:

1) HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
   BP: decomposes
   MP: 282°C
   Solubility in water at 22.5°C: 5.0 μg/L
   Octanol/water partition coefficient: 1.3
   CAS #2691-41-0.

2) RDX (hexahydro-1,3,5-trinitro, 1,3,5-tetrazine)
   BP: decomposes
   MP: 203.5°C
   Solubility in water at 25°C: 60 μg/L
   Octanol/water partition coefficient: 7.5
   CAS #121-82-4.

3) 135TNB (1,3,5-trinitrobenzene)
   BP: decomposes
   MP: 122°C
   Octanol/water partition coefficient: 15
   CAS #25377-32-6.

4) 13DNB (1,3-dinitrobenzene)
   BP: 302°C
   MP: 90°C
   Octanol/water partition coefficient: 31
   CAS #99-63-01.

5) TETRYL (methyl 2,4,6-trinitrophenylnitramine)
   BP: 187°C
   MP: 131°C
   Octanol/water partition coefficient: 43
   CAS #479-45-8.

6) 246TNT (2,4,6-trinitrotoluene)
   BP: 280°C (explodes)
   MP: 80.1°C
   Solubility in water: 130 mg/L
   Octanol/water partition coefficient: 68
   CAS #118-96-7.

7) 24DNT (2,4-dinitrotoluene)
   BP: 300°C (decompose)
   MP: 70°C
   Solubility in water: 300 mg/L
   Octanol/water partition coefficient: 95
   CAS #121-14-2.

8) 26DNT (2,6-dinitrotoluene)
   MP: 66°C
   Octanol/water partition coefficient: 97
   CAS # 606-20-2.

9) NB (nitrobenzene)
   BP: 211°C (Flashpoint 88°C)
   MP: 5.7°C
   Solubility in water: 2 g/L
   Octanol/water partition coefficient: 71
   CAS #98-95-3.

10) 2NT (ortho-nitrotoluene)
    BP: 225°
    MP: -4 to -3°C
    Octanol/water partition coefficient: 171
    CAS #88-72-2.

11) 4NT (para-nitrotoluene)
    BP: 238°
    MP: 54°C
    Octonal/water partition coefficient: 202
    CAS #99-99-0.

12) 3NT (meta-nitrotoluene)
    BP: 231°
    MP: 15°C
    Octonal/water partition coefficient: 263
    CAS #99-08-1.

D. **Reagents and SARMS**:

   1) HMX-SARM quality
   2) RDX-SARM quality
   3) TNB-SARM quality
   4) DNB-SARM quality
   5) TETRYL-SARM quality
   6) TNT-SARM quality
   7) 24DNT-SARM quality
   8) 26DNT-SARM quality
   9) NB-SARM quality
   10) 2NT-Reagent grade
   11) 3NT-Reagent grade
   12) 4NT-Reagent grade
   13) Methanol-HPLC grade
   14) Water-regents grade

IV. **Calibration**

A. **Preparation of Standards**: Standards for each analyte were dried to constant weight in a vacuum desicator in the dark. About 0.1 gm (100 mg) of each dried SARM was weighed out to the nearest 0.1 mg and transferred to individual 100-mL volumetric flasks and diluted to volume with HPLC grade

acetonitrile. Stock standards are stored in a refrigerator at 4°C in the dark. Stock standards are usable for periods up to a year after the date of preparation.

If both 24DNT and 26DNT are to be determined, two separate combined analyte stock standards must be prepared. For stock standard #1, 10.0 mL of the HMX and RDX stock standards and 5.0 mL of the 135TNB, 13DNB, NB, 246TNT and 24DNT stock standards are combined in a 500-mL volumetric flask and diluted to volume with methanol. This solution contains 20,000 μg/L of HMX and RDX and 10,000 μg/L of 135TNB, 13DNB, NB, 246TNT and 24DNT. Stock solution #2 is prepared by combining 10.0 mL of the TETRYL and 5.0 mL of the 26DNT, 2NT, 3NT and 4NT stock solutions in a 500-mL volumetric flask and diluting to volume with methanol. This solution contains 20,000 μg/L of TETRYL and 10,000 μg/L of 26DNT, 2NT, 3NT and 4NT.

A 10.0-mL aliquot of combined stock standard #1 is pipetted into a 100-mL volumetric flask and diluted to volume with methanol, giving a concentration of approximately 2000 μg/L HMX and RDX, and approximately 1000 μg/L of the remaining analytes. This solution will be referred to as Solution A. In a similar fashion, a 10.0-mL aliquot of combined stock standard #2 is diluted to 100 mL with methanol giving a concentration of 2000 μg/L of TETRYL and 1000 μg/L of 26DNT, 2NT, 3NT and 4NT. This solution will be referred to as solution AA.

From Solution A and AA, two identical series of working standard are prepared as described below.

Calibration Standards

| | Aliquot of | Size of | Solution Conc. (µg/L) | |
STD	Solu. A (mL)	Vol. Flask (mL)	HMX, RDX	135TNB, 13DNB, NB 246TNT, and 24DNT
B	25.0	50	1000	500
C	25.0	100	500	250
D	10.0	100	200	100
E	5.0	100	100	50
F	5.0	200	50	25
G	1.0	100	20	10
H	10.0 of E	100	10	5
I	5.00 of E	100	5	2.5

Calibration Standards

| | Aliquot of | Size of | Solution Conc. (µg/L) | |
STD	Solu. AA (mL)	Vol. Flask (mL)	TETRYL	26DNT, 2NT, 3NT and 4NT
BB	25.0	50	1000	500
CC	25.0	100	500	250
DD	10.0	100	200	100
EE	5.0	100	100	50
FF	5.0	200	50	25
GG	1.0	100	20	10
HH	10.0 of EE	100	10	5
II	5.00 of EE	100	5	2.5

B. Initial Calibration: All of the standards are diluted 5/5 (V/V) with water in scintillation vials and well shaken (by hand) before analyzing. Duplicate injections of each standard over the concentration range of interest are made in a random order. Peak areas or peak heights are obtained for each analyte. Retention times for the analytes under these conditions are presented in Table 1.

C. Analysis of Calibration Data: The acceptability of a linear model with zero intercept is assessed using the protocol specified in the USATHAMA QA Program (2nd Edition, March 1987). Experience indicates that a linear model with a zero intercept is appropriate. Thus the slope of the best fit

regression line is equivalent to a response factor that can be compared with values obtained from replicate analyses of a single standard each day.

D. **Daily Calibration**: Standards B and BB, described above, are used for daily calibrations after each are diluted 5/5 (V/V) with water. Standards B and BB can be used for a period of 28 days after preparation. Standards are analyzed in triplicate at the beginning of each day, singly after the last sample of the day, and singly at the midway point of the analysis of each day. Response factors for each analyte are obtained over the course of the day and compared with the response factors obtained for the initial calibration.

The mean response factors for daily calibration must agree within ± 25% of the response factors obtained for the initial calibration for the first seven calibrations. Subsequently, response factors must agree within two standard deviations of the initial calibration. If these criteria are not met, a new initial calibration must be obtained.

V. Certification Testing

Individual analyte certification stock solutions are prepared in an identical manner to that described for the calibration stock standards. As with calibration standards, two sets of certification solutions are required if 26DNT and 24DNT or NB and TETRYL are to be included. Combined analyte stock certification standard solutions are also prepared in the manner described for the combined calibration stock standards.

From the combined certification stock solutions, 25.0-mL aliquots are pipetted into individual 500-mL volumetric flasks and diluted to volume with reagent grade water, giving solutions with concentrations of approximately

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1000 μg/L of HMX, RDX and TETRYL and approximately 500 μg/L of the remaining

analytes.

A series of diluted certification solutions are prepared from these

solutions by diluting with water as follows:

Certification Solutions

Level	Aliquot of Solu. A (mL)	Size of Vol. Flask (mL)	HMX, RDX	135TNB, 246TNT, 24DNT	Certification Solution 1 (μg/L) 13DNB, NB	TETRYL	Certification Solution 2 (μg/L) 26DNT, 2NT, 4NT, 3NT
50X	-	-	1000	500		1000	500
70X	100.0	250	400	200		400	200
10X	50.0	250	200	100		200	100
5X	25.0	250	100	50		100	50
2X	10.0	200	40	20		40	20
1X	5.0	200	20	10		20	10
0.5X	10.0 mL or 5X	100	10	5		10	5

Certification test samples are processed as described below.

VI. Sample/Solution Storage

Combined stock standards should be refrigerated at 4°C, stored in the

dark and be used within 30 days of preparation. Certification solutions and

samples should be prepared the day of the analysis.

VII. Procedure:

A. Sample Preparation: Samples and certification solutions are pre-

pared for analysis by combining a 5.00-mL aliquot with an equal volume of

methanol in scintillation vials, shaking thoroughly and filtering through

0.5-μm Millex-SR filters. The first 3 mL of solution is discarded, and the

remainder is collected in a clean scintillation vial. These filtered solu-

tions will be referred to as sample solutions.

B. Determination: Determination of analyte concentration in the sample solutions is obtained by RP-HPLC-UV at 254 nm. A 100-μL injection loop is flushed with 500 μL of sample solution and injected onto an LC-18 column eluted with 1.5 mL/min of 1:1 V/V methanol-water. Retention times and capacity factors for the analytes of interest and a number of potential interferences are given in Table 1 for both LC-18, the primary column, and LC-CN, the confirmation column. Chromatograms obtained for the primary analytes are shown in Figure 1.[*]

VIII. Calculation

A. Response factors: Since a linear calibration curve with zero intercept is to be expected, calculations of results on a daily basis are obtained using response factors calculated for each analyte. The mean response ($\overline{R}$) for each analyte from repeated determinations of Standards B and BB is obtained in either peak area or peak height units. The response factor (RF) for each analyte is obtained by dividing the mean response by the known concentration (C) in units of μg/L

$$RF = \frac{\overline{R}}{C} \, . \tag{1}$$

B. Analytical Concentration: The concentrations (μg/L) of each analyte (C_a) are obtained by dividing the response for each analyte (R_a) by the appropriate response factor (RF_a)

$$C_a = \frac{R_a}{RF_a} \, . \tag{2}$$

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[*] Editor's note: included here as the bottom portion of Figure 2.

IX. Daily Quality Control

A. __Control spikes__: Spiked water samples are prepared as described for Class 1 method in the USATHAMA QA Program (2nd Edition, March 1987). This requires the use of a method blank, a single spike at two times the certified reporting limit and duplicate spikes at ten times the certified reporting limit for each analytical lot. Control spikes are prepared using the appropriate spiking solution in an identical manner as described in Section V.

B. __Control Charts__: The control charts required are described for Class 1 methods in USATHAMA QA Program (2nd Edition, March 1987). This will require use of standard Shewhart $\overline{X}$ and R charts for the duplicate high spike and moving average $\overline{X}$ and R charts for the single low spike. Details on the charting procedures required are specified in USATHAMA QA Program (2nd Edition, March 1987).